11/16/01 - FD
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WHAT IS CLAIMED IS:

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1. A method for purifying adenovirus from contaminants in a sample pool, comprising:

contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite; and

eluting the bound adenovirus from the hydroxyapatite.

25mM NaCl

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2. The method of claim 1, wherein the sample pool comprises sodium chloride in a concentration of from about 150 to 500 mM.

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3. The method of claim 1, wherein the hydroxyapatite chromatographic medium is equilibrated with a buffer comprising sodium chloride at a concentration of from about 150 to 500 mM before the step of contacting the sample pool with the hydroxyapatite.

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4. The method of claim 1, further comprising the step of washing the hydroxyapatite with a buffer comprising sodium chloride in a concentration of 150 to 500 mM, wherein the hydroxyapatite comprises an adenovirus bound thereto.

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5. The method of claim 1, wherein the adenovirus is eluted using a buffer comprising sodium chloride in a concentration from about 150 mM to 500 mM.

10-300mM NaPO4

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6. The method of claim 1 wherein the sodium chloride concentration in a buffer used in the method is from about 350 mM to 450 mM.

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~~102~~ 7. The method of claim 1, wherein the sample pool is prepared from an eluate of a conventional chromatography medium.

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PURIFIED VIRUS PURIFICATION HYDROXYAPATITE

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ACN53

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GENE THERAPY

8. The method of claim 7 wherein the conventional chromatography medium is:
an anion exchange resin;
an immobilized metal ion affinity resin;
a size exclusion chromatography resin; or
a medium used in hydrophobic interaction chromatography.

9. The method of claim 1, wherein the eluting step is a gradient elution to 600 mM phosphate.

10. The method of claim 1, wherein the eluting step is a step elution with 250 mM phosphate.

11. The method of claim 1, wherein a buffer used in the method comprises glycerol or sucrose.

12. The method of claim 1, wherein the concentration of adenovirus in the sample pool is equal to or less than 1×10^{14} particles per ml.

13. The method of claim 1, wherein the adenovirus comprises a therapeutic gene.

14. The method of claim 1, wherein the adenovirus is ACN53.

15. The method of claim 1, wherein the adenovirus comprises a nucleic acid sequence from the p53 gene or from the p21 gene.

16. A method of claim 1, which reduces the concentration of a contaminant in the sample pool by at least 80%.

17. A method of claim 1, which reduces the concentration of empty capsids by at least 75%.

18. A method of claim 1, which reduces the concentration of BSA by at least 70%.

19. A method for purifying adenovirus from contaminants in sample pool, comprising:

i) contacting the sample pool with a hydroxyapatite chromatographic medium to reversibly bind the adenovirus to the hydroxyapatite;

ii) washing the adenovirus-bound hydroxyapatite with a buffered solution; and

iii) eluting the bound adenovirus from the hydroxyapatite,

wherein the sample pool is a buffered solution comprising about 50 mM sodium phosphate pH about 7.5, about 400 mM sodium chloride, about 2% sucrose, about 2 mM $MgCl_2$, and about 10% glycerol, and the concentration of total contaminants is reduced by at least 80%.